

# GENETIC DIVERSITY OF OLD KLADRUBER AND NONIUS HORSE POPULATIONS THROUGH MICROSATELLITE VARIATION ANALYSIS

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## ABSTRACT

The aim of this study was to evaluate the genetic diversity across horse populations of Old Kladruber and Nonius breeds and to assess the degree of subdivision based on genetic relationships within them. In total, 270 Czech Old Kladruber and 18 Nonius horses from Slovak studs have been included in study. To determine the level of genetic variability the dataset of 17 microsatellites has been used. The average values of observed heterozygosity ( $0.68 \pm 0.03$ ) and gene diversity ( $0.66 \pm 0.02$ ) indicated good level of variability across populations. The value of FIS ( $0.04 \pm 0.02$ ) showed relative balanced proportion of homozygotes and heterozygotes and signalized the existence of HWE across populations. According to the value of Shannon's information index ( $1.48 \pm 0.07$ ) the high degree of overall variability has been confirmed. Most of the variations in dataset explained the differences among individuals within each population. The FST index and Nei's distances showed stronger connection between the Old Kladruby black variety and the Nonius, compared to the grey variety. The segregation of Nonius and Old Kladruber populations demonstrated also results of PCA analysis.

**Key words:** horses, breeds, Old Kladruber, Nonius, genetic diversity, genetic variability, microsatellite markers

## 1 INTRODUCTION

The management of genetic diversity within population is a key factor in any breed conservation programme for protecting the animal genetic resources (Gupta *et al.*, 2015). Domestication of livestock species including horse populations and long history of migration, selection, and adaptation within them have created an enormous variety of breeds (Groeneveld *et al.*, 2010). Over the last several centuries, more than 400 distinct horse breeds have been established by genetic selection and have held a valuable place within other livestock species through service in war, agriculture, sport and companionship (McCue *et al.*, 2012). Changes of conditions in production systems as well as significant competition among breeds

create expectations of risks, which will negatively influence their surviving and can result in the loss of variability through stochastic sampling, particularly when the number of reproducing individuals is restricted (Kasarda and Kadlečík, 2007; Winton *et al.*, 2015).

Based on the fact that the ancient old Spanish horses are extinct, the Old Kladruber breed is unique but also endangered by the loss of diversity, mainly due to the historical bottlenecks and intensive inbreeding (Petlachová *et al.*, 2012; Janova *et al.*, 2012). The Old Kladruber horse originates from old Spanish and Italian bloodlines in the sixteenth and seventeenth century. It was bred as the native karst horse, and during succeeding generations it was crossed with the old Neapolitans and horses of Spanish descent obtained from Spain, Germany, and

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Denmark (Lynghaug, 2009). The Old Kladruber horse has also played a part in the development of other horse breeds, for example Nonius and Lipizzan. The Kladruby horses were bred at Mezöhegyes stud in Hungary, and were one of the bases of the Nonius breed which takes name from its foundation sire a French stallion known as Nonius Senior. Born in Normandy in 1810, Nonius Senior was a mixed breed, with Norfolk Roadster and Norman blood. Nonius Senior was bred to mares of several different breeds, and his descendants were interbred similarly with Spanish Neapolitan horses and later, for further refinement, with English Thoroughbreds. Today, the steady and stoutly built Nonius is used under saddle and in harness (Reeve and Bigs, 2011).

The comprehensive information about genetic diversity and population structure is highly important to evaluate the essential outlines for any appropriate conservation and sustainable management of breeding programs to preserve maximal genetic variability and future adaptation potential of breeds (Khanshour *et al.*, 2015; Winton *et al.*, 2013). The molecular genetic studies of diversity regarding the horse populations are largely based upon microsatellite markers which offer advantages that are particularly appropriate for conservation projects (Bordonaro *et al.*, 2012). Microsatellites are used as highly informative markers in population analysis because they allow the detection of distorting events in the population (selection, migrations, random drift) as well as the degree of inbreeding (Dovč *et al.*, 2006).

The aim of this study was to determine the level of genetic diversity based on microsatellite variability within and across populations of Old Kladruber and Nonius horses. Besides that the degree of potential populations' subdivision and relationship resulting from historical connectedness between the breeds have been evaluated.

## 2 MATERIAL AND METHODS

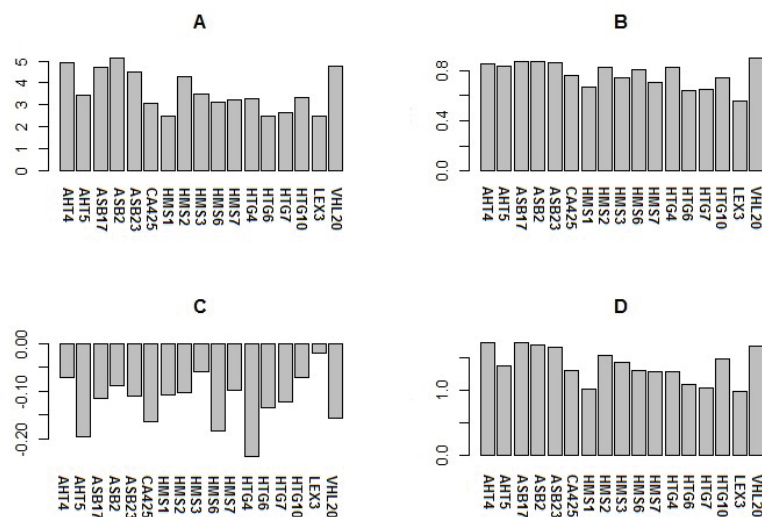
In this study the genotyping data obtained from 238 horses of two breeds, Old Kladruber (270) originating from Czech Republic and Nonius (18) from Slovak studs were evaluated. For description of population subdivision within the Old Kladruber horses two colour varieties have been considered as separate populations (grey variety, 175 animals; black variety, 95 animals). To determine the level of genetic variability within and across each selected horse population the dataset of 17 microsatellite loci (*AHT4*, *AHT5*, *ASB17*, *ASB2*, *ASB23*, *CA425*, *HMS1*, *HMS2*, *HMS3*, *HMS6*, *HMS7*, *HTG4*, *HTG6*, *HTG7*, *HTG10*, *LEX3*, and *VHL20*), recommended primarily for paternity testing by ISAG (International Society for Animal Genetics) has been used.

The characterization of genetic diversity state within populations was realised by the calculation of following parameters: the mean number of alleles (MNA), observed heterozygosity ( $H_o$ ), gene diversity expressed as expected heterozygosity ( $H_e$ ), effective allele number ( $A_{ne}$ ) and Shannon's information index (I) using Genalex version 6.1 (Peakall and Smouse, 2006, 2012). The departure from the Hardy-Weinberg equilibrium (HWE) resulting from significance of differences between observed and expected genotype frequencies was tested by the Chi-square test. The amount of inbreeding-like effect across ( $F_{IT}$ ) and within ( $F_{IS}$ ) populations was evaluated according to Weir and Cockerham (1984). An analysis of molecular variance (AMOVA) estimating the genetic structure indices using information on the allelic content of haplotypes, as well as their frequencies stored entered as a matrix of Euclidean squared distances, was performed using 10,000 permutations with Arlequin v3.5 software (Excoffier *et al.*, 2005). The genetic differentiation among populations reflected relationship within individuals as well as populations were estimated based on the Nei's distance and Wright's  $F_{ST}$  index using Genalex version 6.1. Subsequently, the subdivision of each population was tested based on the principal components analysis (PCA) adopted in R package Adegnet (Jombart and Ahmed, 2011) and visualised by R software v 3.2.2 (R Core Team, 2013).

## 3 RESULTS AND DISCUSSION

Across selected populations and analysed microsatellite markers the total number of alleles 132 was found. The numbers of alleles ranged from 5 (*HMS1*) to 12 (*ASB17*) and was in average  $7.76 \pm 0.45$ . The effective allele numbers ranged from 6.85 (*VHL20*) to 1.99 (*HTG6*). The value of observed average heterozygosity ( $0.68 \pm 0.03$ ) indicated a good level of variability across individuals. The  $H_o$  was in range from 0.5 (*HTG6*) to 0.86 (*VHL20*). Based on gene diversity the same state of variability was found. The  $H_e$  varied from 0.49 (*HTG6*) to 0.85 (*VHL20*). The sufficient proportion of heterozygous animals was indicated also by  $F_{IS}$  index. Across all loci the average  $F_{IS}$  value close to zero ( $0.04 \pm 0.02$ ) indicated relatively balanced proportion of heterozygotes vs. homozygotes and also signalise the HWE in populations. However, using Chi-square test the departure from HWE due to the significance of differences between observed and expected genotype frequencies ( $P < 0.05$ ) was identified at up to 12 loci (*AHT4*, *AHT5*, *ASB17*, *ASB23*, *HMS2*, *HMS3*, *HMS6*, *HMS7*, *HTG6*, *HTG7*, *LEX3*, *VHL20*).

According to Shannon's information index the locus *VHL20* ( $I = 1.96$ ) was considered as the most in-



**Figure 1:** Distribution of effective allele numbers (A), observed heterozygosity (B), Wright's FIS index (C) and Shannon's information index (D) per locus (in order AHT4, AHT5, ASB17, ASB2, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, LEX3, and VHL20) across analysed population.

formative. The average value of I index across all loci ( $1.48 \pm 0.07$ ) also reflected high degree of overall genetic variability within populations, because the Shannon's information index generally reflected the effectiveness of microsatellite markers to reveal the genetic variations. Figure 1 shows the distribution of four basic genetic parameters describing the state of diversity across selected horse populations for each locus. Overall, all of the applied genetic diversity parameters expressed good level of genetic variability within evaluated populations (Table 1) and indicated the state of biodiversity comparable with studies assessed Hungarian Noric population (Mihók *et al.* 2009), Czech Old Kladruber (Vostrý *et al.*, 2011) and also other horse breeds with Austro-Hungarian origin (Achmann *et al.*, 2004; Dovč *et al.*, 2006).

The AMOVA analysis has been applied to evaluate the hierarchical population structure and to explain the proportion of differences affected by the subdivision of populations. The results showed that the most of the variation was explained by the differences conserved among individuals within each population (84 %). The subdivision of populations according to their origin reflected up to 9 % of the genetic diversity in the analysed dataset and

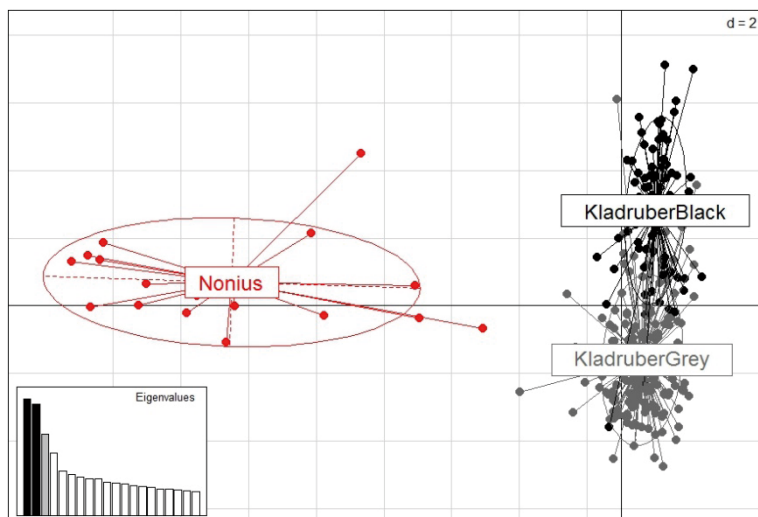
7 % of total genetic variability was partitioned within individuals in whole population.

The obtained values of  $F_{ST}$  index and Nei's genetic distances as frequently used indicators of relatedness significantly confirmed the connectedness between analysed populations resulting from their historical origin. According to the generally accepted criteria the average value of  $F_{ST}$  at level 0.08 can be regarded as low and the populations as only slightly genetically differentiated. But the  $F_{ST}$  values at level 0.03 also indicated expected closest genetic similarity between both the Old Kladruber populations. Based on our results, the connection between the Old Kladruby black variety to the Nonius seems to be relatively stronger (0.05) compared to the grey variety (0.07), which showed also subsequent evaluation of Nei's genetic distances. At the intra-population level of Old Kladruber the  $D_A$  value 0.17 was found. The highest differentiation was identified between the Old Kladruber grey variety and population formed by the Nonius horses ( $D_A = 0.41$ ).

The clear segregation of Nonius and Old Kladruber populations was demonstrated also based on PCA analysis. Moreover, this analysis showed the separation of grey

**Table 1:** The average values of genetic diversity parameters obtained across microsatellites

Population	$A_{ne}$	I	Ho	He	F
Nonius	$4.05 \pm 0.28$	$1.46 \pm 0.08$	$0.82 \pm 0.02$	$0.73 \pm 0.02$	$-0.39 \pm 0.05$
Old Kladruber black	$3.48 \pm 0.29$	$1.40 \pm 0.07$	$0.67 \pm 0.04$	$0.68 \pm 0.03$	$0.02 \pm 0.02$
Old Kladruber grey	$3.31 \pm 0.26$	$1.32 \pm 0.08$	$0.65 \pm 0.04$	$0.66 \pm 0.03$	$0.01 \pm 0.04$



**Figure 2:** Representative results of PCA analysis applied to the evaluations of populations' subdivision

and black Old Kladruber colour variety (Fig. 2). First two principal components were sufficient for the explanation of the genetic structure and division of individuals into the groups. The PC1 component explained 18.52 % of the variation, while the second component explained 7.97 % of the variability.

Preservation of endangered species is one of the most important goals for the present biological sciences, especially in the context of natural ecosystem stability. In the case of horse, conservation programs are usually initiated for breeds which present a unique genetic and phenotypic value (Mackowski *et al.*, 2015). An understanding of the genetic characteristics of a population is paramount in order to inform and implement conservation strategies that maintain genetic diversity (Winton *et al.*, 2013). The baseline molecular analysis provides a dependable tool which can be used together with the quantitative approach and traditional breeding strategies for an efficient design of preservation strategy. Moreover, the genetic distances can be used for explanation of the population structure and genetic distinctiveness of breeds (Gupta *et al.*, 2015).

#### 4 CONCLUSION

Our study showed that the level of biodiversity conserved within both analysed horse populations estimated using 17 microsatellite systems, is sufficient. The genetic differentiations between the Old Kladruber and Nonius population clearly reflected the formation of separate groups in accordance to their breeding history. The results also showed that the connection between the Old Kladruber black variety to the Nonius seems to be rela-

tively stronger compared to the grey variety that reflected mainly the breeding background of Nonius horses. Alongside pedigree analyses, morphological measures, and immunogenetics the microsatellites can provide a tool for the improvement of conservation strategy and current breeding programme to maintain maximal genetic variability and future adaptation potential of both breeds.

#### 5 ACKNOWLEDGEMENT

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